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Preface

Dear Participants,

Confronted with the ever-increasing complexity of technical processes and the growing demands on their efficiency, security and flexibility, the scientific world needs to establish new methods of engineering design and new methods of systems operation. The factors likely to affect the design of the smart systems of the future will doubtless include the following:

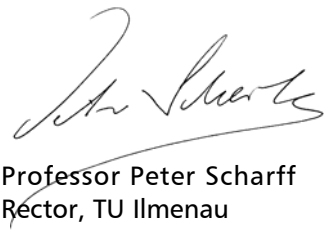
- As computational costs decrease, it will be possible to apply more complex algorithms, even in real time. These algorithms will take into account system nonlinearities or provide online optimisation of the system's performance.
- New fields of application will be addressed. Interest is now being expressed, beyond that in "classical" technical systems and processes, in environmental systems or medical and bioengineering applications.
- The boundaries between software and hardware design are being eroded. New design methods will include co-design of software and hardware and even of sensor and actuator components.
- Automation will not only replace human operators but will assist, support and supervise humans so that their work is safe and even more effective.
- Networked systems or swarms will be crucial, requiring improvement of the communication within them and study of how their behaviour can be made globally consistent.
- The issues of security and safety, not only during the operation of systems but also in the course of their design, will continue to increase in importance.

The title "Computer Science meets Automation", borne by the 52nd International Scientific Colloquium (IWK) at the Technische Universität Ilmenau, Germany, expresses the desire of scientists and engineers to rise to these challenges, cooperating closely on innovative methods in the two disciplines of computer science and automation.

The IWK has a long tradition going back as far as 1953. In the years before 1989, a major function of the colloquium was to bring together scientists from both sides of the Iron Curtain. Naturally, bonds were also deepened between the countries from the East. Today, the objective of the colloquium is still to bring researchers together. They come from the eastern and western member states of the European Union, and, indeed, from all over the world. All who wish to share their ideas on the points where "Computer Science meets Automation" are addressed by this colloquium at the Technische Universität Ilmenau.

All the University's Faculties have joined forces to ensure that nothing is left out. Control engineering, information science, cybernetics, communication technology and systems engineering – for all of these and their applications (ranging from biological systems to heavy engineering), the issues are being covered.

Together with all the organizers I should like to thank you for your contributions to the conference, ensuring, as they do, a most interesting colloquium programme of an interdisciplinary nature.
I am looking forward to an inspiring colloquium. It promises to be a fine platform for you to present your research, to address new concepts and to meet colleagues in Ilmenau.



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A 2-Compartment Model of Glutamine and Ammonia Metabolism in Liver Tissue

7 NEW METHODS AND TECHNOLOGIES FOR MEDICINE AND BIOLOGY

Introduction

The aim of this modelling study was to develop an adequate model of glutamine and ammonia metabolism in perfused rat livers under particular consideration of the metabolic zonation in the liver lobulus.

The liver is the main organ of intermediary metabolism. It consists of thousands of identical small sub-units, the liver lobuli. Each lobulus consists of a central vein and is confined by portal fields in a hexagonally arrangement. From each portal field, the blood flows into the lobulus and is drained through the central vein. The area surrounding the portal field is called the periportal region and the area around the central vein is called the perivenous region. During passage from the portal field to the central vein, blood passes a number of hepatocytes. Hepatocytes realize almost all metabolic functions, e.g. synthesis and degradation of amino acids, proteins, lipids and carbohydrates [1]. Due to highly regulated enzyme expression [2], most metabolic pathways are only present either in the periportal or the perivenous compartment of the lobulus. This is called metabolic zonation. As a consequence, synthesis and degradation are simultaneously carried out but in different compartments of the lobuli. One example where this metabolic zonation has an important impact is glutamine and ammonia metabolism. In the organism toxic ammonia is removed by converting glutamate into glutamine. The glutamine-rich blood enters the liver at the periportal region and the enzyme glutaminase (G) degrades glutamine to ammonia and glutamate. Subsequently, toxic ammonia is eliminated by the enzyme carbamoyl-phosphate synthetase (C) in the course of urea synthesis [1, 3]. Remaining ammonia is detoxified in periportal hepatocytes by converting glutamate into glutamine by the glutamine synthetase (GS).

Experimental Data

The model identification presented here is based on experimental data published in [3]. In [3], the metabolic zonation was investigated in perfused rat livers by comparing metabolic flux rates depending on the perfusion direction. The isolated rat livers were perfused without recirculation either in physiological antegrade (from portal to central vein) or in retrograde (from central to portal vein) direction with a weight-specific constant perfusion flow F_{mL}. For several experiments, either glutamine synthetase or carbamoyl-phosphate synthetase were inhibited by addition of methionine sulfoximine to the perfusion fluid or by using a bicarbonate-free buffer, respectively. Modelling is based on measured glutamine (GLN), ammonia (NH4), urea (UREA) and glutamate (GLU) concentrations in the influent and effluent of the perfused rat livers recorded from 16 perfusion experiments (Fig. 2, blue bars). All data were obtained in metabolic steady-state and are means from 4-12 separate runs. Data preprocessing was performed by scaling measured effluent concentrations in order to guarantee that the overall sum of nitrogen in the influent together with the estimated endogenously arising nitrogen is equal to the overall sum of nitrogen in the effluent.

Knowledge- and Data-driven Modelling

Model development comprises the knowledge-driven determination of the model structure and the subsequent data-driven estimation of the model parameters.

The model consists of eight differential equations with one equation for each concentration $c_{GLN,i}$, $c_{NH4,i}$, $c_{UREA,i}$, $c_{GLU,i}$ and for each compartment (i = 1,2), respectively. Both compartments are run through by the perfusion flow F carrying the concentrations $c_{j,Input}$ in influent (j = GLN, NH4, UREA, GLU). During antegrade perfusion, the effluent concentrations $c_{j,Output}$ are equal to the concentrations $c_{j,2}$ in compartment 2 (Fig. 1), while during retrograde perfusion, they are equal to the concentrations $c_{j,1}$ in compartment 1 (not shown).

The periportal compartment 1 with the volume V_1 includes glutamine degradation by glutaminase and urea synthesis by carbamoyl-phosphate synthetase. In contrast, the perivenous compartment 2 with the volume V_2 contains glutamine synthesis by the enzyme glutamine synthetase (Fig. 1). The corresponding reaction equations consider stimulation (Michaelis-Menten equation with Michaelis-Menten constant K_M) and inhibition (noncompetitive inhibition with dissoziation constant K_I) by substrates and

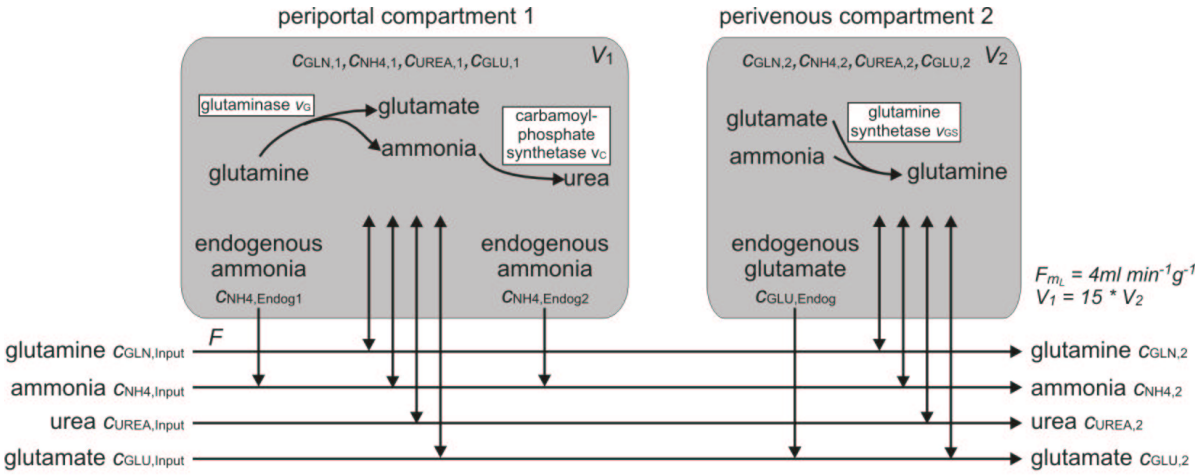
products. Thus, glutamine degradation v_G has a maximum velocity $v_{MAX,G}$ and is stimulated by glutamine ($K_{M,G,GLN}$) and portal ammonium ions ($K_{M,G,NH4}$) [3, 4]. Urea synthesis v_C is characterized by the maximum velocity $v_{MAX,C}$ and the stimulation by ammonia ($K_{M,C,NH4}$). Glutamine synthesis v_{GS} has the maximum velocity $v_{MAX,GS}$ and is stimulated by ammonia ($K_{M,GS,NH4}$) and glutamate ($K_{M,GS,GLU}$). Furthermore, it is inhibited by the product glutamine ($K_{I,GS,GLN}$) [4]. Consequently, each differential equation can consist of three parts. The first part describes concentration changes caused by synthesis and degradation from the three metabolic reactions (G,C,GS) that were taken into account. The both other parts describe the change of concentrations caused by the inflow and the outflow of the perfusion stream F.

Endogenous ammonia arises from endogenous protein and amino acid breakdown [5]. Endogenous ammonia production is located mainly in the periportal compartment, which consists of more than 95 % of all hepatocytes of the glutamine/NH4 system. Modelling the observed effects caused by endogenous ammonia requires two positions where the endogenous ammonia portions $c_{NH4,Endog1}$ and $c_{NH4,Endog2}$ operate. Furthermore, several experiments point to an endogenous source of glutamate $c_{GLU,Endog}$ in the perivenous compartment 2 (Fig. 1).

During experiments with inhibition of glutamine synthesis or urea synthesis, the respective metabolic reactions are only active to a very small degree. In order to model this effect, the synthesis rates v_{GS} and v_C are multiplied with the additional activity factors e_{GS} and e_C. During the simulation of experiments without inhibition, theses factors are set to one and, thus, have no influence on the synthesis rates. In case of simulating experiments with inhibition, the activity factors possess values much smaller than one and, therefore, decrease the synthesis rates.

A common model for all 16 experiments was identified. The parameters of the model were estimated by one of the following methods: (i) Parameters are known or can be calculated from known experimental settings. (ii) Parameters can be directly calculated from the measurement data. (iii) Parameters are optimized by fitting the model output to the measurement data using a nonlinear optimization technique.

The first method is applied for the yield coefficients Y and the terms F/V_i that are constant for all experiments due to the weight-specific perfusion flow F_{mL}. The second method is used to estimate the total levels of endogenous ammonia and glutamate. All remaining model parameters are optimized by minimizing the root square error (RSE)



periportal compartment 1

$$dc_{GLN,1}/dt = -1/Y_G \cdot v_G - F/V_1 \cdot c_{GLN,1} + F/V_1 \cdot c_{GLN,Input}$$

$$dc_{NH4,1}/dt = v_G - 1/Y_C \cdot v_C \cdot e_C - F/V_1 \cdot c_{NH4,1} + F/V_1 \cdot (c_{NH4,Input} + c_{NH4,Endog1})$$

$$dc_{UREA,1}/dt = v_C \cdot e_C - F/V_1 \cdot c_{UREA,1} + F/V_1 \cdot c_{UREA,Input}$$

$$dc_{GLU,1}/dt = v_G - F/V_1 \cdot c_{GLU,1} + F/V_1 \cdot c_{GLU,Input}$$

perivenous compartment 2

$$dc_{GLN,2}/dt = v_{GS} \cdot e_{GS} - F/V_2 \cdot c_{GLN,2} + F/V_2 \cdot c_{GLN,2}$$

$$dc_{NH4,2}/dt = -1/Y_{GS} \cdot v_{GS} \cdot e_{GS} - F/V_2 \cdot c_{NH4,2} + F/V_2 \cdot (c_{NH4,1} + c_{NH4,Endog2})$$

$$dc_{UREA,2}/dt = - F/V_2 \cdot c_{UREA,2} + F/V_2 \cdot c_{UREA,1}$$

$$dc_{GLU,2}/dt = -1/Y_{GS} \cdot v_{GS} \cdot e_{GS} - F/V_2 \cdot c_{GLU,1} + F/V_2 \cdot (c_{GLU,1} + c_{GLU,Endog})$$

$$v_G = v_{MAX,G} \cdot c_{GLN,1}/(K_{M,G,GLN} + c_{GLN,1}) \cdot c_{NH4,Input}/(K_{M,G,NH4} + c_{NH4,Input})$$

$$v_C = v_{MAX,C} \cdot c_{NH4,1}/(K_{M,C,NH4} + c_{NH4,1})$$

$$v_{GS} = v_{MAX,GS} \cdot c_{NH4,2}/(K_{M,GS,NH4} + c_{NH4,2}) \cdot c_{GLU,2}/(K_{M,GS,GLU} + c_{GLU,2}) \cdot K_{I,GS,GLN}/(K_{I,GS,GLN} + c_{GLN,2})$$

Fig. 1: Structure of the 2-compartment liver model (antegrade perfusion)

between the measured and preprocessed concentrations c_j^k and the simulated model output $c_{j,Output}^k$ for experiments k (k = 1,...,16 and j = GLN, NH4, UREA).

It should be mentioned, that the model output $c_{GLU,Output}$ can not be adapted with an acceptable quality to the measured glutamate concentrations. It is assumed, that in contrast to the GLN, NH4 and UREA release, the glutamate level is strongly influenced by other pathways not taken into account here. However, the model can simulate the effects of the three considered metabolic reactions on the release of glutamate.

Results

The modelling results are shown in Fig. 2. The 2-compartment model developed is able to cover all main effects with respect to glutamine, ammonia and urea release observed during the experiments with changing perfusion direction. The following effects are described in [3] and are captured by the model:

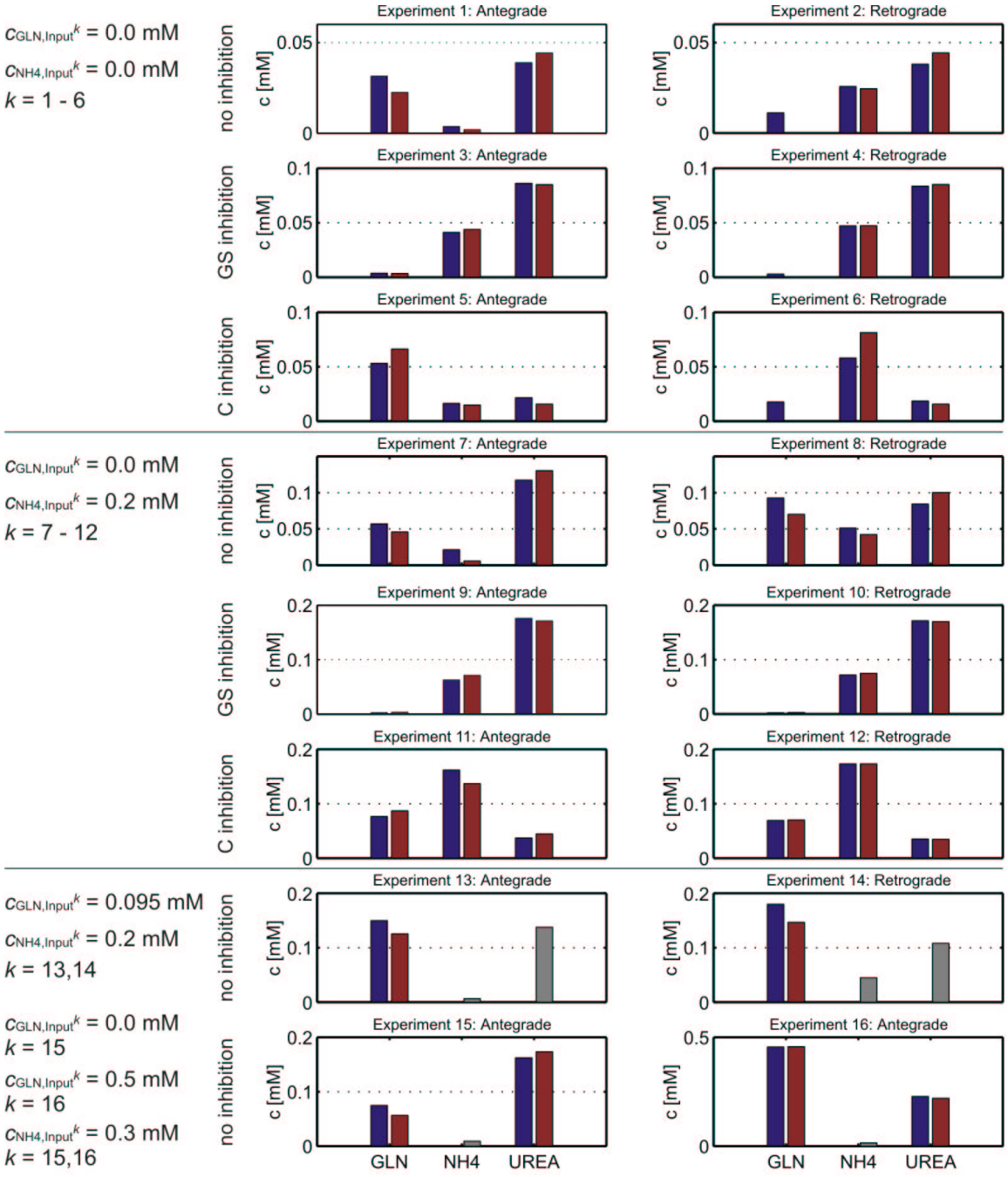


Fig 2: Results of the 2-compartment model (RSE = 0.356); blue = measurement data, red = model output, gray = model output, but no measurement data available

- Ammonium ions can be very efficiently eliminated during antegrade perfusion but are released by the liver to a considerable extend during retrograde perfusion (Exp. 1 and 2, Exp. 7 and 8 in Fig. 2).
- Endogenous ammonium ions are utilized for synthesis of glutamine during antegrade perfusion but are washed out during retrograde perfusion (Exp. 5 and 6).
- Added ammonium ions are mainly converted to urea during antegrade perfusion but are converted to glutamine and urea during retrograde perfusion (Exp. 7 and 8).
- During the inhibition of the competing pathways urea and glutamine synthesis, the

direction of perfusion does not influence the release of glutamine or urea (Exp. 3 and 4, Exp. 9 and 10, Exp. 11 and 12).

- During physiological antegrade perfusion, urea synthesis can remove high ammonia concentrations but is restricted to levels above approximately 0.05 *mM* (Exp. 3 and 9). The subsequent glutamine synthesis removes very efficiently low ammonia concentrations which were not eliminated by urea synthesis (Exp. 1 and 7). This feature is obtained by the Michaelis-Menten constants with $K_{M,C,NH4} >> K_{M,GS,NH4}$.

Conclusions

Comparisons of these results with a 1-compartment model show that this simpler model is structurally unable to cover the observations made during the perfusion experiments. That means, the 1-compartment model (even if including the same metabolic reactions as the 2-compartment model) can not be fitted to the measurement data with an acceptable modelling error. Consequently, an adequate liver model of glutamine and ammonia metabolism requires the structural mapping of metabolic zonation. By using expert knowledge and experimental data, a 2-compartment model covering all main observed effects could be developed. This 2-compartment model can be used as a general tool for simulation, e.g. defects in metabolic reactions that decrease the ammonia uptake.

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